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Postganglionic Site of Action of Nicotine with Special Reference to its Direct Action on Blood Vessels.

HENRY HAIMOVICI.*

From the Medical Services and Laboratories of The Mount Sinai Hospital, New York City.

In the intact animal, administration of small amounts of nicotine is known to cause amongst other effects, constriction of the arteries of the ear and of the pads, a large rise of blood pressure, erection of hairs, dilatation of the pupil.[1] In an isolated organ, such as a strip of small intestine, nicotine induces inhibition of its movements, similar to those observed in the intact animal.[2]

These effects are attributed to the stimulating action of nicotine on the autonomic cells[3] and/or to its action on the adrenal medulla, the latter resulting in augmented epinephrine secretion.[4] In both instances, *i.e.* the intact animal and the isolated organs, it is assumed that nicotine acts only indirectly on the effector cells.

However, using the Laewen-Trendelenburg preparation in the frog, which is essentially an isolated vascular bed, we have previously reported that nicotine causes vasoconstriction.[5] This fact is in agreement with that presented by other investigators.[6,7] Since the presence of ganglion cells on peripheral blood vessels has been denied by most authors[8,9] it appeared to us that, in addition to its known stimulation of autonomic ganglion cells, nicotine may also have a more peripheral action. The present study was thus undertaken to investigate the postganglionic locus of action of nicotine with special reference to its direct action on blood vessels.

Methods and Material. The Laewen-Trendelenburg (L-T) preparation in frogs (*Rana pipiens*) was used as previously described.[5] Frog Tyrode solution was used as a perfusate. Two Mariotte bottles were connected by a Y-piece with the perfusion system, *i.e.* the rubber tubing leading to the aortic cannula. One bottle contained plain Tyrode solution. The other contained, dissolved in the perfusate, one of the following substances: nicotine base (Eastman Kodak), curare (Intocostrin, Squibb and Sons), tetraethylammonium hydrochloride (Etamon),† N,N, dibenzyl-beta-chloroethylamine hydrochloride (Dibenamine).‡

In addition to the "perfusion method", the effects of nicotine were also tested by the "bio-assay method" using the direct injection into the glass cannula inserted into the abdominal aorta. The vasomotor action of the different drugs was measured in terms of output variations of drops per minute.

Results. (A). Effect of nicotine I). Injection of small amounts of nicotine: the bio-assay technic. As previously reported, the injection of a small amount of nicotine (1-10 γ) in the L-T preparation elicits 2 major reactions: (a) Twitchings of the thigh and leg muscles, lasting as a rule from 30 to 60 seconds. (b) A marked reduction (50%-80%) of the outflow from the abdominal vein, as

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1 Langley, J. N., *J. Physiol.*, 1918-19, **52**, 247.

2 Langley, J. N., and Magnus, R., *J. Physiol.*, 1905, **33**, 34.

3 Langley, J. N., *J. Physiol.*, 1901, **27**, 224.

4 Cannon, W. B., Aub, J. C., and Binger, C. A. L., *J. Pharm. and Exp. Therap.*, 1911-12, **3**, 379.

5 Haimovici, H., and Pick, E. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 234.

6 Handovski, H., and Pick, E. P., *Arch. f. Exp. Path. u. Pharmakol.*, 1913, **71**, 89.

7 Loewi, O., *J. Physiol. U. S. S. R.*, 1937, **22**, 390.

8 Woolard, H. H., Heart, 1926, **13**, 319.

9 Stoehr, P., Jr., *Mikrosk. Anat. d. veget. Nervensyst., Berlin, Springer*, 1928, 67.

† Etamon was supplied by Parke, Davis and Co.

‡ Dibenamine, Trademark of Givaudan-Delawanna, Inc. This drug was supplied by the Givaudan-Delawanna Co., Inc., Delawanna, N. J., through the courtesy of Dr. W. Gump.

expressed in terms of drops per minute. The duration of the vasoconstrictor effect varied, the average duration ranging between 15 and 30 minutes, until the output returned to its initial level. Nicotine tested several times in the same preparation induced regularly the same vasoconstrictor effect. Tachyphylaxis to nicotine was rarely noted. It was found, however, that some frogs exhibited individual variations as to the doses of nicotine and exceptionally some were entirely refractory to the usual small doses. In view of the latter fact, each individual preparation was first tested for its sensitivity to nicotine, before any further test was undertaken.

II) *Perfusion of the L-T preparation with nicotine.* 1. Continuous perfusion of the frogs vessels with a solution of nicotine in a concentration ranging from 1:250,000 to 1:50,000 induced: (a) Twitchings of the muscles lasting in most instances, several minutes. Exceptionally, twitchings were absent. (b) Marked vasoconstriction (50%-60%). The onset of the latter effect did not coincide with the start of the infusion. A delay of 2 to 4 minutes was noted. The maximum vasoconstriction appeared 5 to 10 minutes after the beginning of the nicotine perfusion. After 40 to 60 minutes the output returned, in most instances, to its initial level, despite continuous nicotine perfusion (Fig. 1). Additional injection of large amounts of nicotine (100γ-500γ)

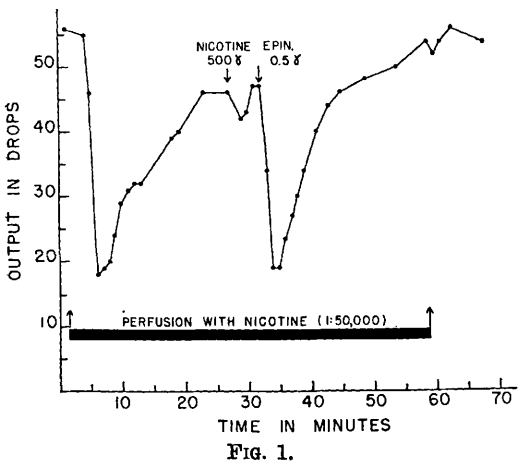

FIG. 1.

Self-blocking effect of nicotine. The L-T preparation becomes insensitive to additional large amounts of nicotine, while it retains its sensitivity to epinephrine.

remained practically without any effect. It is noteworthy that at this phase, the preparation still exhibited its usual sensitivity to the vasoconstrictor effect of epinephrine (0.5 γ to 1 γ). The self-blocking effect of nicotine, as observed in the prolonged perfusion of the L-T preparation, appears to be similar to the action of this alkaloid on other tissues. Indeed, nicotine in high concentrations produces a contracture of the rectus abdominis of the frog followed by relaxation, and then the muscle remains insensitive to further addition of nicotine.[10] The paralysis of the tissues induced by high concentrations of nicotine seems to be a characteristic toxic manifestation of this alkaloid. 2. Perfusion for 3 to 10 minutes only, with concentrations of nicotine as above, induced identical patterns of vasoconstriction. In the same preparation, repeated perfusions with nicotine for short durations elicited the same degree of vasoconstriction. The self-blocking phenomenon of nicotine was thus obviated by reducing the perfusion time. The effects of nicotine in this instance were thus similar to those observed with the bio-assay technic.

III) Excision of both sympathetic chains and resection of all mixed nerves of the hind legs in the L-T preparation did not alter the action of nicotine on the blood vessels in both the bio-assay and the perfusion methods.

(B). *Effect of nicotine after tetraethylammonium hydrochloride* (*T.E.A.*) Because of the ability of T.E.A. ion to block the action of "nicotinic-stimulating" substances,[11,12] it seemed desirable to test the effect of nicotine after its administration. T.E.A. was used in concentrations ranging from 1:10,000 to 1:25,000. Its perfusion through the frog's hind legs was not followed by any vasomotor effect *per se*. The vasoconstrictor effect of either nicotine or epinephrine remained unaltered by the perfusion with T.E.A. in preparations both with and without the sympathetic ganglia (Fig. 2). In perfusions with T.E.A.

10 Gasser, H. S., *Physiol. Rev.*, 1930, **10**, 35.

11 Acheson, G. H., and Moe, G. K., *J. Pharm. and Exp. Therap.*, 1946, **87**, 220.

12 Acheson, G. H., and Pereira, S. J., *J. Pharm. and Exp. Therap.*, 1946, **87**, 273.

lasting over an average of 30 minutes the vasoconstrictor effect of nicotine was slightly impaired. However, a larger dose of nicotine (*i.e.* 10 γ instead of 5 γ used in the control test) exhibited the same ability to induce marked vasoconstriction. Muscular twitchings usually induced by nicotine were abolished in most cases by T.E.A. but those induced by nerve stimulation remained unaffected by this agent.

(C). *Effect of nicotine after curare.* Langley stated that "in the frog the postganglionic nerves are not exempt from paralysis by curare as they are in the bird and the mammal."[1] It seemed then of interest to test the action of nicotine after administration of curare. Curare, as a d-tubocurarine chloride preparation (Intocostrin, Squibb), was used in Tyrode solution in concentrations ranging from 1:12,500 to 1:50,000. The time of perfusion with curare varied from 3 minutes to over 1 hour. Curarization was considered satisfactory when stimulation of the mixed nerves of the hind legs failed to induce muscular twitchings.

The effects following injection of nicotine, during or after perfusion with curare, varied with the concentration of the latter and the duration of the perfusion. In high concentrations of curare (1:12,500) with time-perfusion

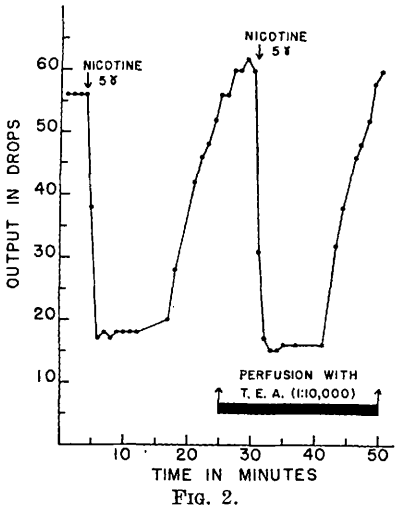

FIG. 2.

Unaltered effect of nicotine after tetraethylammonium hydrochloride in a L-T preparation without sympathetic ganglia.

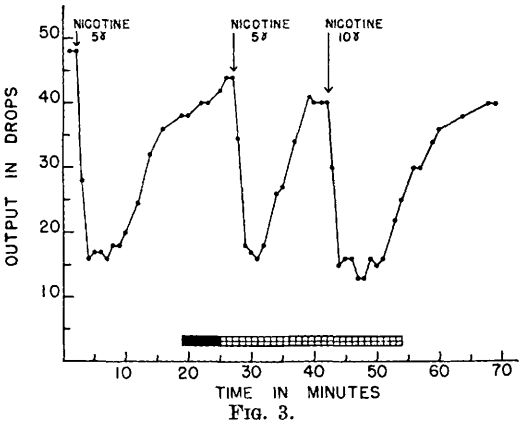

FIG. 3.

Unaltered effect of nicotine after curare. The plain bar represents the perfusion time with curare (Intocostrin, 1:50,000). The cross-hatched bar represents the effective curarization time.

exceeding 10 minutes, nicotine induced only slight vasoconstriction. In lower concentrations (1:50,000) with time-perfusion not exceeding 3 to 10 minutes, nicotine exhibited almost, if not the same degree, of vasoconstriction as that obtained in the control tests (Fig. 3). Care was exercised to ascertain the curarization of the preparation throughout the testing of nicotine.

(D) *Effect of nicotine after Dibenamine.* Dibenamine being a potent and highly specific adrenergic-blocking agent,[13,14] the study of the effect of nicotine on the blood vessels after blocking the transmission of sympathetic postganglionic impulses with this agent seemed of further interest. Dibenamine, in the concentration of 1:250,000 to 1:25,000 while inhibiting the vasoconstrictor action of epinephrine completely within 5 to 10 minutes after the onset of the perfusion, does not influence the action of nicotine. In addition, within 30 to 45 minutes after the onset of the perfusion, when the action of large amounts (50γ-1000γ) of epinephrine is completely blocked, nicotine still induces notable vasoconstriction and visible twitchings of the muscles.[14]

Discussion. From the foregoing data it appears that after excision of both sympathetic

[13] Nickerson, M., and Goodman, L. S., *J. Pharm. and Exp. Therap.*, 1947, **89**, 167.

[14] Haimovici, H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 486.

chains and resection of all spinal nerves of the frog's hind legs in the L-T preparation, nicotine induces the same degree of vasoconstriction as before. This seems to indicate that in this preparation the locus of action of nicotine is not in the autonomic ganglia but more peripheral. In order to test this interpretation it remained to demonstrate that the hind legs of the frog were ganglion-cell free. Both pharmacologic and histologic methods were used to that end.

The T.E.A. ion is known to be capable of blocking the stimulating action upon ganglion cells by "nicotinic-stimulating" substances.[15,16] Since the perfusion of the hind legs with this agent did not abolish the nicotinic vasoconstriction it appears that nicotine acts beyond the ganglion cells.

The action of nicotine was further investigated after blocking the postganglionic sympathetic transmission with curare and Dibenamine. Curare, in the frog, unlike in the birds and mammals, extends its action to postganglionic autonomic fibers. From the above data it appears that despite effective curarization, nicotine still continued to exhibit its vasoconstrictor action.

With Dibenamine, an adrenergic-blocking agent, similar observations were made. It is of interest to note that De Vleeschhouwer[17] reported "that 10 to 15 mg/kg Dibenamine do not reverse the hypertensive action of nicotine in the atropinized dog. Larger quantities decrease slightly, but never reverse, the vasopressor effects of nicotine." The observations made in the intact animal seem to confirm our own observations made in the L-T preparation that nicotine is also capable of acting beyond the postganglionic apparatus of the autonomic nervous system.

The ability of nicotine to act directly on blood vessels without synaptic intervention is shown by the fact that it also induces vasoconstriction in the isolated rabbit's ear.[18]

In man, Coon and Rothman[19,20] injected nicotine intradermally and noted pilomotor activity and sweating. These authors believe that nicotine in this instance acted through an axon reflex mechanism within the skin. In the light of data presented in this study there is reason, however, to believe that the locus of nicotine action was more directly on the neuroeffector cells.

The validity of the interpretation of the above pharmacologic findings is further supported by our histologic study of the blood vessels of the frog's hind legs. Numerous serial sections of sciatic, femoral and tibial vessels and the corresponding nerves of 10 hind legs were studied. No ganglion cells were encountered.§ These findings in the frog are thus in agreement with previous reports concerning mammalian blood vessels of the extremities.[8,9] It is of interest to mention, however, the presence of abundant perivascular melanophore cells in the frog. The physiologic significance of the latter is not clear as yet. There are no known connections between nerve fibers and the chromatophores. As is well known, the pituitary secretion exercises a direct effect upon these pigment cells. Shen[21] has shown that while nicotine provokes an intense and prolonged melanophore-expanding action upon normal pale frogs, it fails to do so in hypophysectomized frogs or in isolated legs. It appears, therefore, that the vasoconstrictor action of nicotine observed in the L-T preparation is quite unrelated to the perivascular melanophore cells.

Summary and Conclusion. In the L-T preparation, which is essentially an isolated vascular bed, nicotine induces marked vasoconstriction. Resection of both sympathetic chains and of all spinal nerves does not alter the action of nicotine. T.E.A. ion, capable of blocking the action of the "nicotinic-stimulating" substances upon ganglion cells, curare,

15 Burn, J. H., and Dale, H. H., *J. Pharm. and Exp. Therap.*, 1914, **6**, 417.

16 Hunt, R., *J. Pharm. and Exp. Therap.*, 1926, **28**, 367.

17 De Vleeschhouwer, G. R., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 151.

18 Pick, E. P., Personal communication.

19 Coon, J. M., and Rothman, S., *J. Pharm. and Exp. Therap.*, 1940, **68**, 301.

20 Coon, J. M., and Rothman, S., *J. Pharm. and Exp. Therap.*, 1941, **73**, 1.

§ I am indebted to Dr. Frederick G. Zak for his help in this study.

21 Shen, T. C. R., *J. Physiol.*, 1937, **90**, 518.

capable of paralyzing postganglionic fibers, and Dibenamine capable of blocking the same fibers, do not abolish the vasoconstrictor action of nicotine. Serial sections of the vessels of the frog's hind legs have shown the absence of ganglion cells.

It appears, therefore, that in the L-T preparation the site of action of nicotine is peripheral to the postganglionic fibers, possibly directly on the blood vessels. In the intact animal, it may be assumed that in addition to its known sites of action on ganglion cells, and adrenal medulla, nicotine may also act directly on the neuroeffector cells of the blood vessels.

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Effects of Intrathecal Administration of Epinephrine on Cardiac Rhythm in Dogs Anesthetized with Cyclopropane.

J. P. HOWARD, LOUIS LEVY II, AND JOHN ADRIANI.

From Department of Surgery, Louisiana State University and Department of Cardiology, Charity Hospital of Louisiana, New Orleans.

Recently there has been a trend in clinical anesthesia to combine epinephrine with local anesthetic drugs to prolong the duration of spinal anesthesia. At times it is necessary to supplement spinal anesthesia with a rapidly acting drug such as cyclopropane. It is well established that cyclopropane increases cardiac irritability and this increased irritability is further enhanced by epinephrine. In dogs, as little as .01 mg of epinephrine per kilo intravenously precipitates ventricular premature beats, tachycardia or fibrillation during deep cyclopropane anesthesia. There is some evidence that vasopressors do not quickly pass from the subarachnoid space into the blood stream. Bray, Katz, and Adriani[1] have shown that epinephrine, neosynephrine, ephedrine and oenethyl cause no vasopressor response when administered intrathecally in therapeutic doses in man. Inasmuch as so little epinephrine causes serious circulatory changes, it is important to know whether or not the passage of epinephrine from the subarachnoid space into the general circulation is sufficiently rapid to be a hazard under such circumstances.

Method. Observations were made on 6 dogs. Thirty mg of nembutal per kilo was administered intraperitoneally to facilitate the insertion of an endotracheal tube. Cyclopropane and oxygen were administered by the carbon dioxide absorption technique to the point of intercostal paralysis and maintained at this level. After 20 minutes of cyclopropane anesthesia control electrocardiograms were obtained using lead II.

The first dog was given the standard test dose of .01 mg kilo body weight in 5 cc normal saline intravenously until ventricular tachycardia occurred. Meek[2] *et al.* have found that dose produces reflex vagal inhibition of the pacemaker with or without escape of the A-V node bundle or ventricle but without tachycardia or fibillation. With cyclopropane this same dose causes ventricular premature beats, tachycardia or fibrillation. After the rhythm had returned to normal 1 mg of epinephrine was administered intrathecally. One milligram is the upper limit of the dose usually employed clinically. Continuous electrographic recordings were made prior, during and immediately after the injection. The dog was observed for 1 hour, at the end of which time the standard test dose of epinephrine was repeated intravenously. The second dog was prepared as the first except that at the end of 1 and 2 hours 2 mg and 5 mg of epinephrine

[1] Bray, K., Katz, S., and Adriani, J., in press.

[2] Meek, W. J., Hathaway, H. R., and Orth, O. S., *J. Pharm. and Exp. Therap.*, 1937, **61**, 240.